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The goal of this project has been to develop and characterize an electrochemical microsystem for the rapid point-of-care genetic screening of breast-cancer. We introduced new electrical DNA biosensing routes for genetic screening of breast-cancer. These include novel nanoparticle-based bioassays, label-free schemes based on the intrinsic electroactivity of DNA and coding protocols for multi-target DNA detection. By addressing the major challenge of signal amplification, our research has led to major improvements in the sensitivity of electrical biosensing of DNA segments specific to breast-cancer gene BRCA1. These new advances have been coupled withnew schemes for minimizing non-specific adsorption and discriminating against non-complementary sequences. Such coupling of high sensitivity, specificity, and multi-target detection capabilities permits electrical DNA assays to rival the most advanced optical protocols. The new particle-based detection and coding technologies offer great promise for developing fast, simple, and user-friendly DNA sensing devices for point-of-care breast-cancer testing.

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Table of Contents

Introduction

Wide-scale genetic testing requires the development of fast-responding low-cost miniaturized analytical devices. Traditional methods for detecting DNA hybridization are too slow and labor intensive. Biosensors offer a promising alternative for simpler, faster, and cheaper DNA assays. Hybridization biosensors commonly rely on the immobilization of a single-stranded (ss) oligonucleotide probe onto a transducer surface to recognize - by hybridization - its complimentary target sequence.

Electrochemical devices have received considerable attention in the development of sequence-specific DNA hybridization biosensors. Such devices rely on the conversion of the DNA base-pair recognition event into a useful electrical signal. The high sensitivity of electrical devices, coupled to their compatibility with modern microfabrication technologies, portability, low cost (disposability), minimal power requirements, and independence of optical pathway or sample turbidity, make them excellent candidates for decentralized DNA testing. Direct electrical reading of DNA hybridization thus offers great promise for developing simple, rapid, and easy-to-use, cost-effective DNA sensing devices (in a manner analogous to pocket-size blood-glucose meters).

The goal of this research has been to develop and characterize a miniaturized biosensing system for decentralized genetic screening of breast-cancer. The realization of rapid point-of-care DNA testing requires proper attention to major challenges of high sensitivity and selectivity, multi-target detection, and integration of the sample preparation with the actual DNA detection on a single microchip flow platform. Such challenges have been met by designing innovative biosensor strategies that would allow testing for breast cancer to be performed more rapidly, inexpensively, and reliably in a decentralized setting.

Body

This report summarizes our activity over the 4-year (7/00-6/04) period. In accordance to our original objectives our research focused on various fundamental and practical aspects of electrical detection of DNA segments specific to the breast-cancer gene BRCA1. As described in this section, we have made a substantial progress, and introduced innovative nanoparticle-based electrochemical routes for improving the reliability of devices for genetic screening of breast-cancer (based on new label-free and particle-based amplification schemes). This 3.5 year activity has resulted in 16 research papers (published or in press in major journals; see attached list) and several invited presentations in major meetings. (Several more publications are expected in the late part of 2004.) Such findings pave the way to major improvements in electrical detection of DNA and offer innovative routes for simple, rapid, and user-friendly devices for breast-cancer screening.

Signal Amplification for Detecting Low Target Concentrations. Nanoparticle-based materials offer excellent prospects as ultrasensitive tags for electrical sensing of DNA. We examined several amplification processes for enhancing the sensitivity of particle-based electrical DNA assays, including catalytic enlargement of the metal tracer, and use of polymeric microbeads carrying multiple redox tracers externally (on their surface) or internally (via encapsulation). Combined with additional amplification units and processes,

such bead-based multi-amplification protocols meet the high sensitivity demands of electrochemical DNA breast-cancer biosensors.

We have demonstrated a triple-amplification bioassay, coupling the carrier-sphere amplifying units (loaded with numerous gold nanoparticles tags) with the 'built-in' preconcentration of the electrochemical stripping detection and catalytic enlargement of the multiple gold-particle tags (1). The gold-tagged beads were prepared by binding biotinylated metal nanoparticles to streptavidin-coated polystyrene spheres. Such triple-amplification route offered a dramatic enhancement of the sensitivity, to allow detection of DNA targets down to the 300amol level. Factors affecting the performance have been optimized. Such amplified electrical transduction offers great promise for ultrasensitive detection of other biorecogntion events. An analogous amplification route, involving polymeric carrier spheres loaded with numerous CdS nanoparticles tags, is currently being examined in connection to the detection of cadmium.

Internal encapsulation electroactive tags within carrier beads offers another an attractive alternative to their external loading. For example, we developed an ultrasensitive electrical DNA detection based on polystyrene beads impregnated with a redox marker (2). The resulting 'electroactive beads' are capable of carrying a huge number of the ferrocenecarboxaldehyde marker molecules and hence offer a remarkable amplification of single hybridization events. This allowed chronopotentiometric detection of target DNA down to the 5.1×10^{-21}mol level (~31,000 molecules) in connection to 20 min hybridization and 'release' of the marker in an organic medium. The dramatic signal amplification advantage is combined with a remarkable discrimination against a huge excess (10^7) of non-complementary nucleic acids.

We designed a bioelectronic protocol for detecting DNA hybridization based on preparing the metal marker along the DNA backbone (instead of capturing it at the end of the duplex) (3). The new protocol is based on DNA-template induced generation of conducting nanowires as a mode of capturing the metal tag. The use of DNA as a metallization template has evoked substantial research activity directed to the generation of conductive nanowires and the construction of functional circuits. Yet, the DNA-templated assembly of metal wires has not been exploited for detecting DNA hybridization. The new detection scheme consists of the vectorial electrostatic 'collection' of silver ions along the captured DNA target, followed by hydroquinone-catalyzed reductive formation of silver aggregates along the DNA skeleton , along with dissolution and stripping detection of the nanoscale silver cluster. The new protocol thus combines the inherent signal amplification of stripping analysis with effective discrimination against nonhybridized DNA. It yielded a low detection limit of around 100ng/mL, which corresponds to 5 ng in the 50 μL hybridization solution. We also demonstrated for the first time the use of binary inorganic nanoparticles, such as CdS colloids, for electrochemical monitoring of DNA hybridization (4). The new protocol combines the amplification features of nanoparticle/polynucleotides assemblies and highly sensitive stripping potentiometric detection of cadmium, with an effective magnetic isolation of the duplex. The high sensitivity and selectivity make this protocol a useful addition to the armory of nanoparticle-based electrochemical genetic testing schemes.

Multi-Target DNA Detection. We designed an electrochemical coding technology for the simultaneous detection of multiple DNA targets based on nanocrystal tags with diverse redox potentials (5). Functionalizing the nanocrystal tags with thiolated oligonucleotide probes thus offered a voltammetric signature with distinct electrical hybridization signals for the corresponding DNA targets. The position and size of the resulting stripping peaks provided the desired identification and quantitative information, respectively, on a given target DNA. The multi-target DNA detection capability was coupled to the amplification feature of stripping voltammetry (to yield fmol detection limits) and with an efficient magnetic removal of nonhybridized nucleic acids to offer high sensitivity and selectivity. Up to 5-6 targets can thus be measured simultaneously in a single run in connection to ZnS, PbS, CdS, InAs, and GaAs semiconductor particles. Conducting massively parallel assays (in microwells of microtiter plates or using multi-channel microchips, with each microwell or channel carrying out multiple measurements) could thus lead to a high-throughput operation.

We demonstrated also a dual target electrochemical DNA detection based on the use of different enzyme tags (6). The two enzyme tracers selected for the initial demonstration of the dual-target detection, alkaline phosphatase (ALP) and β-galactosidase (GAL), liberate a wide range of phenolic products with different redox potentials. The dual target detection capability has been coupled with high sensitivity and effective discrimination against noncomplementary nucleic acids. The influence of relevant experimental variables was examined and optimized.

Label-Free Electrical Detection. Earlier in the project we introduced a new label-free gene-sensing scheme based on the intrinsic electroactivity of DNA (7,8). Changes in the guanine oxidation process accrued from the hybridization event have thus been exploited for detecting DNA sequences related to the BRCA1 breast cancer gene. The advantages of such direct label-free hybridization measurements have been combined with the magnetic 'removal' of unwanted constituents that commonly hamper such assays. The efficient magnetic separation has thus been shown extremely useful for discriminating against unwanted constituents, including a large excess of co-existing mismatched and non-complementary oligomers, chromosomal DNA, RNA and proteins. A renewable (pencil) graphite electrode was employed for transducing the DNA hybridization event (8). Further signal amplification of such label-free detection was accomplished via the dramatically enhanced accumulation of purine nucleobases in the presence of copper ions. Such protocol involved hybridization of the target to inosine-substituted oligonucleotide probes (captured on magnetic beads), acidic dipurinization of the hybrid DNA, and adsorptive chronopotentiometric stripping measurements of the free nucleobases in the presence of copper ions.

Towards Microchip Devices. We are currently working on coupling of our particle-based DNA assays with chip platforms. Recently we demonstrated a new low-cost user-friendly approach for rapid prototyping of polymeric microfluidic chip devices, based on atmospheric pressure molding (9). The new method brings significant simplification of the process of fabricating PMMA devices, without compromising the quality and accuracy of the channel replication. Present efforts are aimed at integrating sample-handling processes, including sample collection, DNA extraction, reagent mixing and amplification, with the particle-based hybridization detection.

Key Research Accomplishments

During the four years of this project we introduced innovative routes for improving the reliability of electrical devices for genetic screening of breast-cancer. In particular, we have successfully designed new amplification protocol for enhancing the sensitivity of electrochemical bioassays, introduced new simplified label-free protocols (based on the intrinsic electroactivity of DNA) and new coding protocols for simultaneous detection of multiple breast-cancer related sequences. The importance of these new bioassays is reflected from 16 publications in leading international journals. Such developments (combined with our use of magnetic separations for addressing the issues of mismatch discrimination or non-specific adsorption) should facilitate the realization of instant point-of-care breast-cancer testing. The successful realization of such testing will rely on the transformation of the new label-free and particle-based protocols into miniaturized flow systems.

Reportable Outcomes

Papers accepted or published:

a. "Pencil-based renewable Biosensor for Label-free Electrochemical Detection of DNA Hybridization', J. Wang and A. Kawde, Anal. Chim. Acta, 431(2000)219.

b. "DNA Biosensors and Gene Chips", J. Wang, Nucleic-Acid Research (Invited Review), 28(2000)3011.

c. "Magnetic-beads based Label-Free Electrochemical Detection of DNA Hybridization", J. Wang, A. Nasser, A. Erdem, M. Salazare, Analyst, 126(2001)2020.

d. "Amplified Label-Free Detection of DNA Hybridization", J. Wang, and A. Kawde, Analyst, 127(2002)383.

e. "Genomagnetic Electrochemical Assays of DNA Hybridization", J. Wang, D. Xu, R. Polsky, and E. Arzum, Talanta, 56(2002)931.

f. "Magnetically-Induced Solid-State Electrochemical Detection of DNA Hybridization", J. Wang, D. Xu, and R. Polsky, J. Am. Chem. Soc., 124(2002)4208.

g. "Magnetic Field Stimulated DNA Oxidation", J. Wang and A. Kawde, Electrochemistry Communications, 4(2002)349.

h. "Metal-Nanoparticle based Electrochemical Stripping Detection of DNA Hybridization", J. Wang, D. Xu, R. Polsky, and A. Kawde, Anal. Chem. 73(2001)5576.

i. "Silver-Enhanced Colloidal Gold Electrical Detection of DNA Hybridization", J. Wang, R. Polsky, D. Xu, Langmuir, 17(2001)5739.

j. "Electroactive Beads For Ultrasensitive DNA Detection", J. Wang, R. Polsky, A. Merkoci, and K. Turner, Langmuir, 19(2003)989.

k. "Electrochemical Detection of DNA Hybridization based on DNA-Tempated Assembly of Silver Cluster", J. Wang, O. Rincon, R. Polsky, and E. Dominquez, Electrochem. Communications, 5(2003)83.

l. "Dual Enzyme Electrochemical Coding for Detecting DNA Hybridization", J. Wang, A. Kawde, M. Musameh and G. Rivas, Analyst 127(2002)1279.

m. "Electrochemical Stripping Detection of DNA Hybridization based on CdS Nanoparticle Tags", J. Wang, G. Liu, and R. Polsky, Electrochemistry Communications, 4(2002)819.

n. "Amplified Electrical Transduction of DNA Hybridization based on Polymeric Beads Loaded with Multiple Gold Nanoparticle tags", J. Wang and A. Kawde, Electroanalysis, 16(2004)101.

o. "Nanoparticle-based Electrical DNA Assays", J. Wang, Anal. Chim. Acta, **(Invited/Special Issue 500)** 500(2003)247.

p. "Carbon Nanotubes Modified Glassy Carbon Electrodes for Amplified Detection of DNA Hybridization", J. Wang, A. Kawde and M. Mustafa, Analyst, 128(2003)912.

Invited Presentations:

a. "Particle-based DNA Assays", Pittsburgh Conference (March 2003).

b. "Particle-based DNA Assay", Sensors 03, Paris (June 2003).

c. "Particle-based DNA Assay", Bioelectrochemistry, Florence (June 2003).

d. "Nanoparticle-based Electrical DNA Assays", Arizona State University (Chem. Dept.), Pheonix (Dec. 2003).

e. "Nanoparticle-based Electrical DNA Assays", MRS Meeting, San Francisco (April 2004).

Conclusions

We have demonstrated novel nanoparticle-based bioassays, label-free schemes based on the intrinsic electrioactivity of DNA and coding protocols for multi-target DNA detection. By addressing the major challenge of signal amplification, our research has led to major improvements in the sensitivity of electrical biosensing of DNA segments specific

to the breast-cancer gene BRCA1. These new advances have been coupled with new schemes for minimizing non-specific adsorption and discriminating against non-complementary sequences. Such coupling of high sensitivity, specificity, and multi-target detection capabilities permits electrical DNA assays to rival the most advanced optical protocols. The new particle-based detection and coding technologies offer great promise for developing fast, simple, and user-friendly DNA sensing devices for point-of-care breast-cancer testing.

The realization of decentralized DNA testing would require additional developmental work. In particular, the coupling of our particle-based DNA assays with chip platforms would accelerate the realization of wide-scale breast-cancer screening. We have recently assembled a microfabrication laboratory that allows us to micromachine the microfluidic devices essential for such chip-based high throughput automated operation. We also developed an effective protocol for low-cost fabrication of disposable plastic microchips. The coupling of these chip platforms with our particle-based DNA assays would accelerate the realization of wide-scale breast-cancer screening. Although the Army project has been completed, we are currently developing a microfluidic system that will integrate various sample-handling processes, including sample collection, DNA extraction, reagent mixing and amplification, with the actual hybridization detection. The new microsystem will rely on the use of magnetic beads not only for removing unwanted constituents, but also for localizing different probes within the microchannels. The probe-bearing beads could thus be reproducibly introduced, kept in place, manipulated, and removed/replaced after each run (in connection to a proper placement and removal of a magnet). Such reversible magnetic localization obviates the need for 'fixing' the probe onto the chips, regenerating it, and for fabricating multiple hybridization sites or special physical 'barriers', and hence would greatly simplify the fabrication and operational requirements.

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5. J. Wang, G. Liu, and A. Merkoçi, J. Am. Chem. Soc., 125(2003)3214.

6. J. Wang, A. Kawde, M. Musameh and G. Rivas, Analyst 127(2002)1279.

7. J. Wang, and A. Kawde, Analyst, 127(2002)383.

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9. A. Muck, J. Wang, M. Jacobs, G. Chen, M. P. Chatrathi, V. Jurka, Z. Výborný, S. D. Spillman, G. Sridharan, and M. J. Schöning Anal. Chem., 76(2004)2290.

PAPERS RESULTING FROM RESEARCH

The following are publications which have resulted from our research

Genomagnetic electrochemical assays of DNA hybridization published in Talanta 56(2002)931-938

Electroactive beads for ultrasensitive DNA detection published in Langmuir 2003 19, 989-991

Metal nanoparticle-based electrochemical stripping potentiometric detection of DNA hybridization published in Anal. Chem 2001 73, 5576-5581

Magnetic bead-based label-free electrochemical detection of DNA hybridization published in Analyst 2001 126, 2020-2024

Dual enzyme electrochemical coding for detecting DNA hybridization published in Analyst 2002 127, 1279-1282

Silver-enhanced colloidal gold electrochemical stripping detection of DNA hybridization published in Langmuir 2001 17, 5739-5741

Electrochemical detection of DNA hybridization based on DNA-templated assembly of silver cluster published in Electrochemistry Communications 2003 5, 83-86

Magnetically-induced solid-state electrochemical detection of DNA hybridization published in J Am Chem Soc 2002 124, 4208-4209